

FIG. 1a

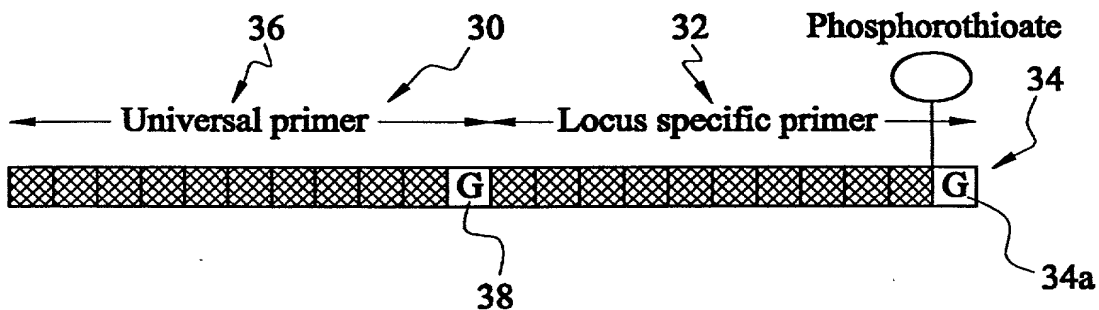


FIG. 2a

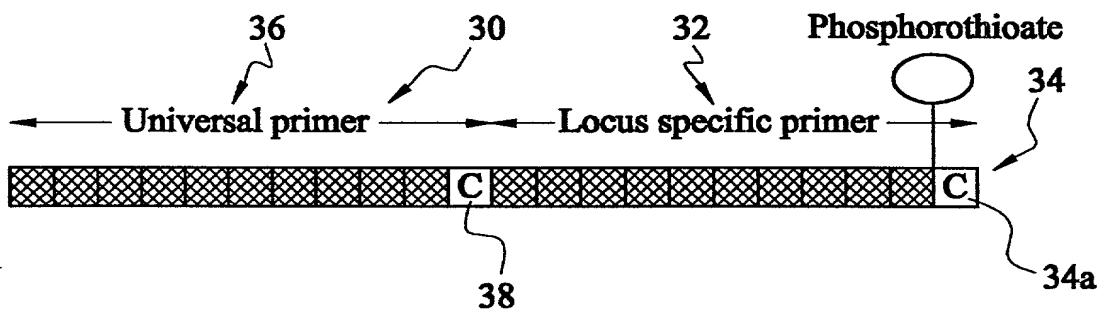


FIG. 2b

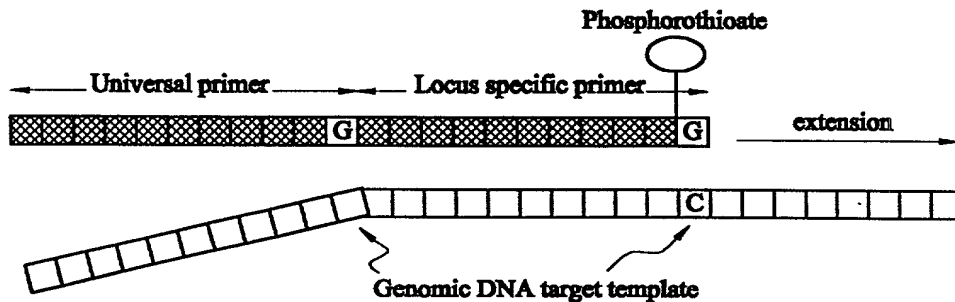
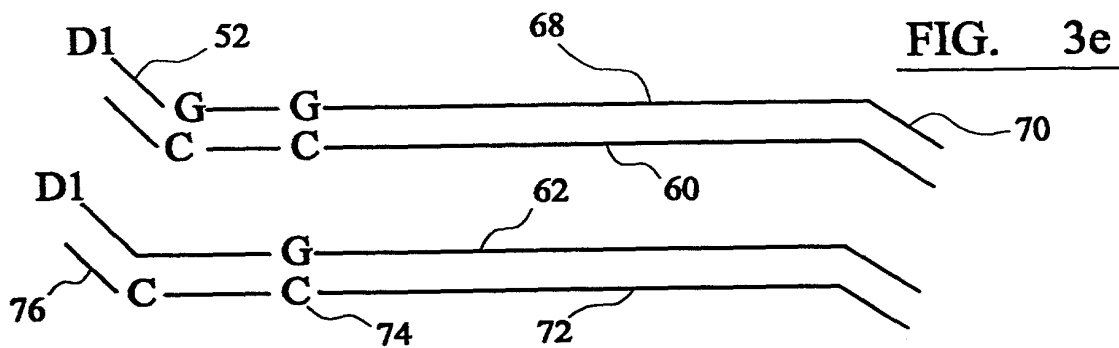
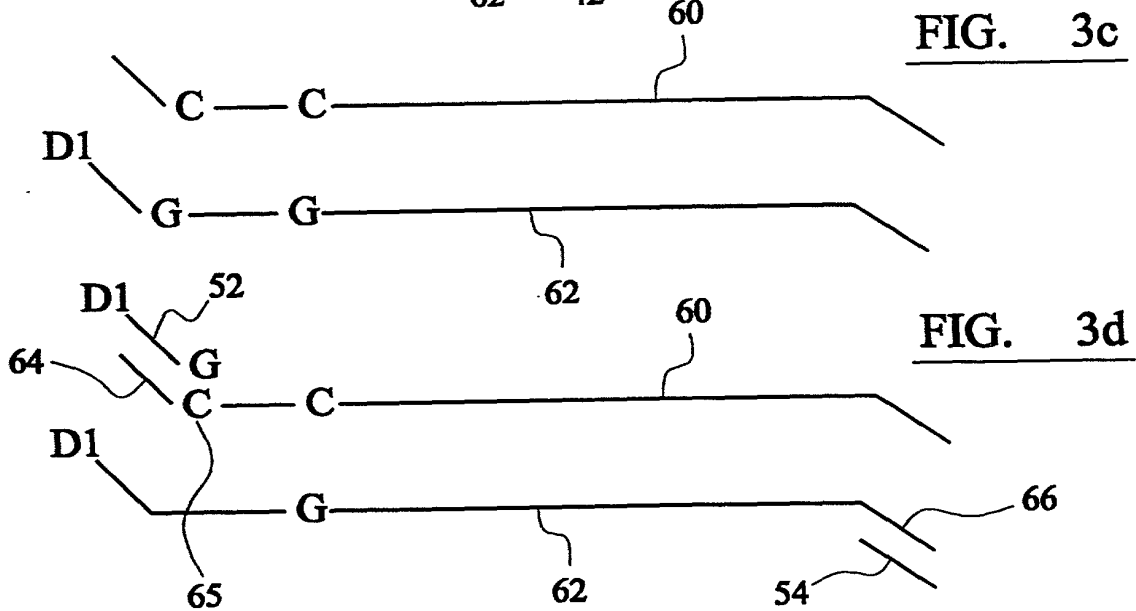
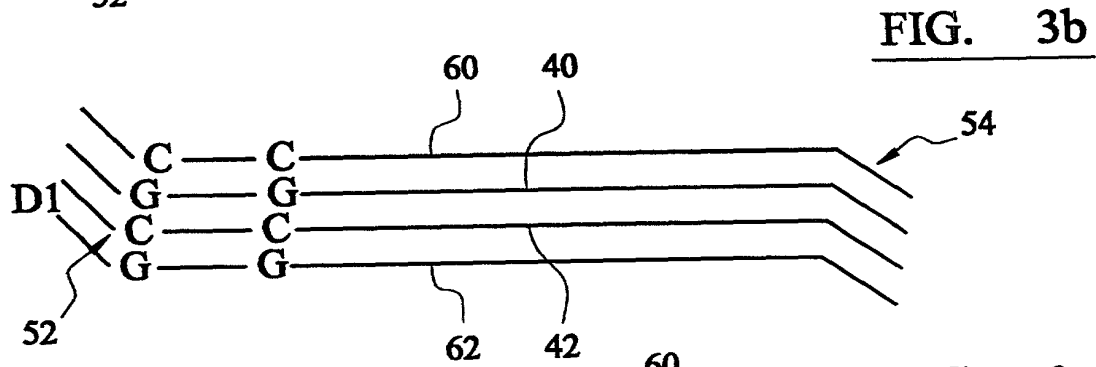
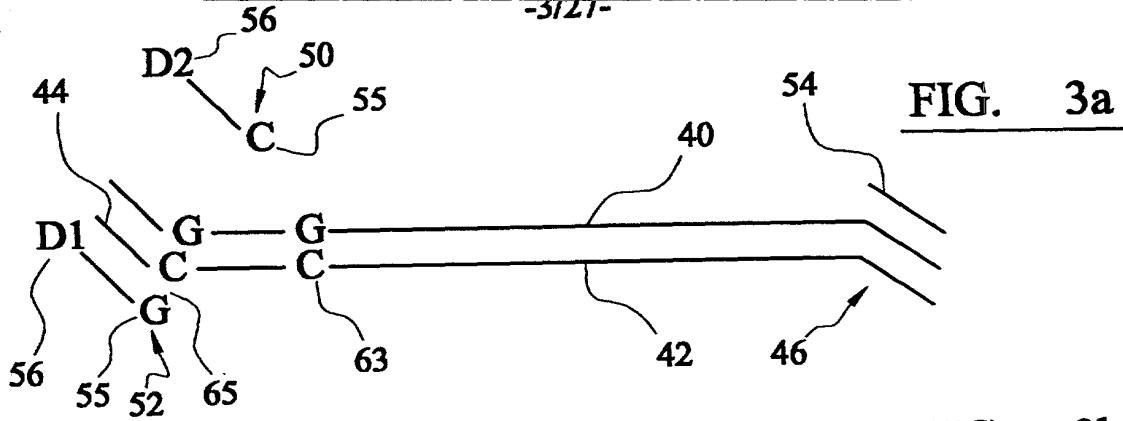


FIG. 6

-3121-



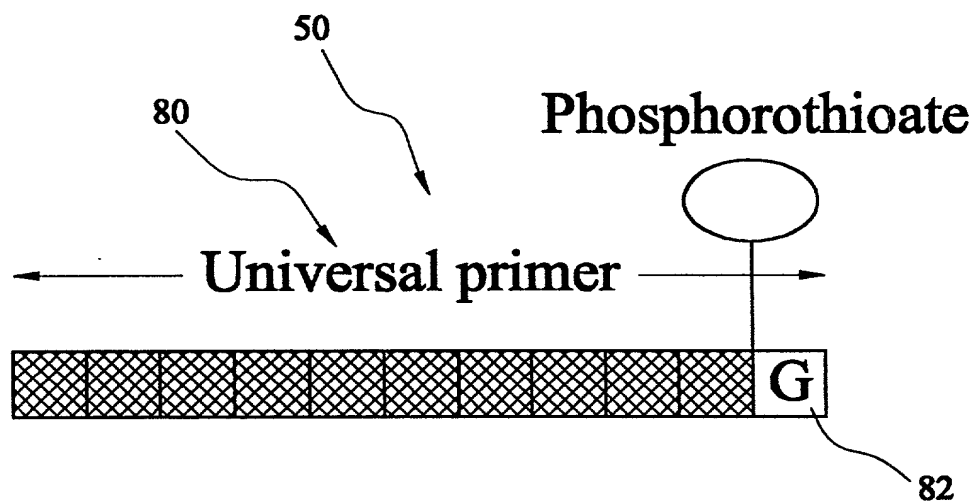


FIG. 4a

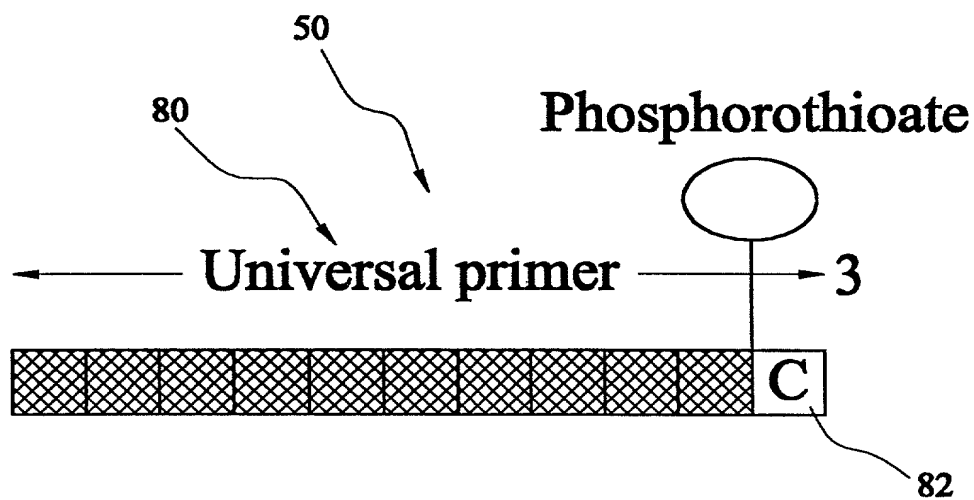


FIG. 4b

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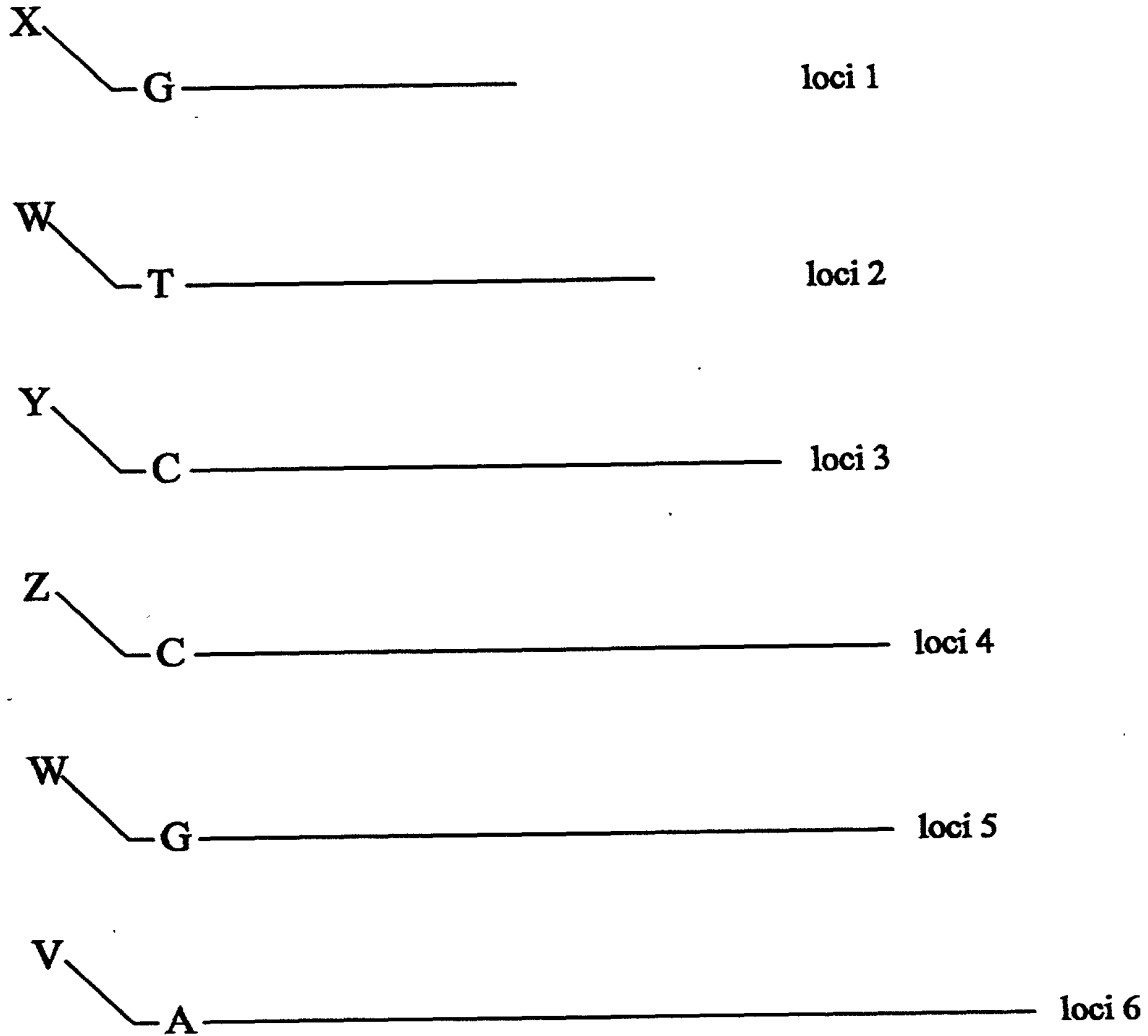
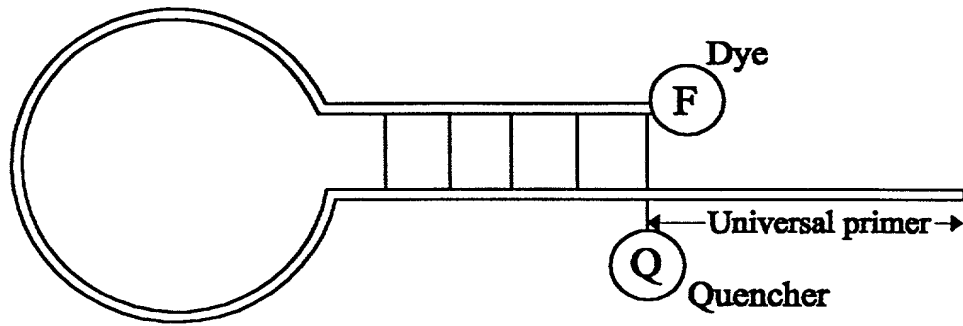


FIG. 5



-----Beacon-----

F= dye label(FAM or JOE) ; ‡ = Quencher - Methyl red; R = AG or TC

FIG. 7a



FIG. 7b

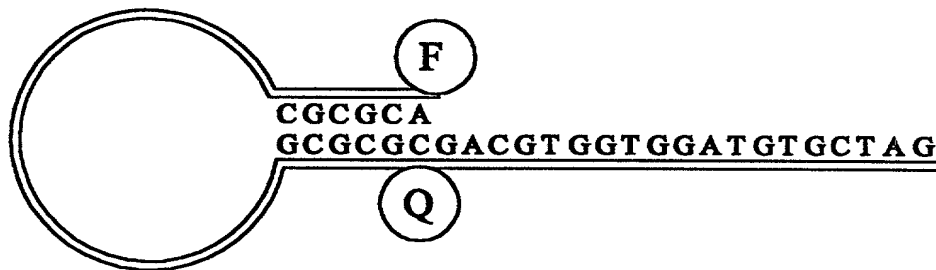


FIG. 7c

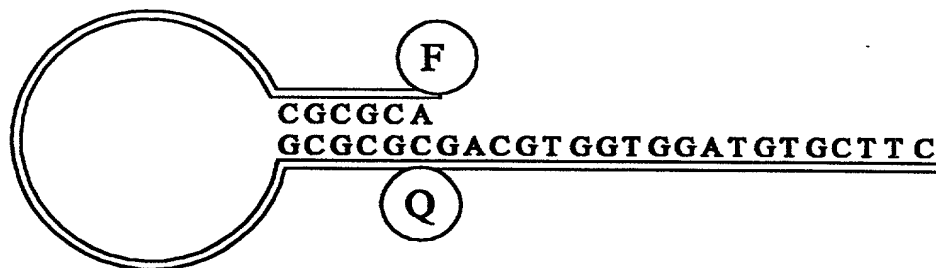
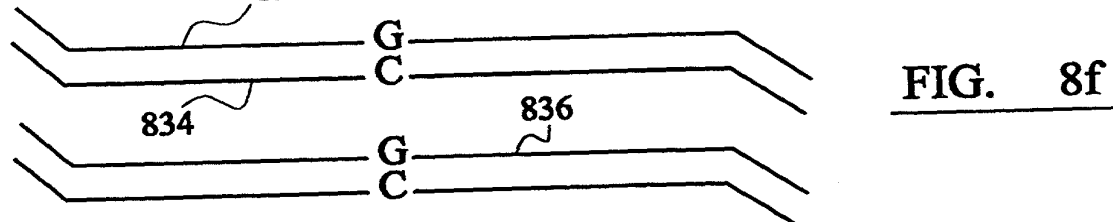
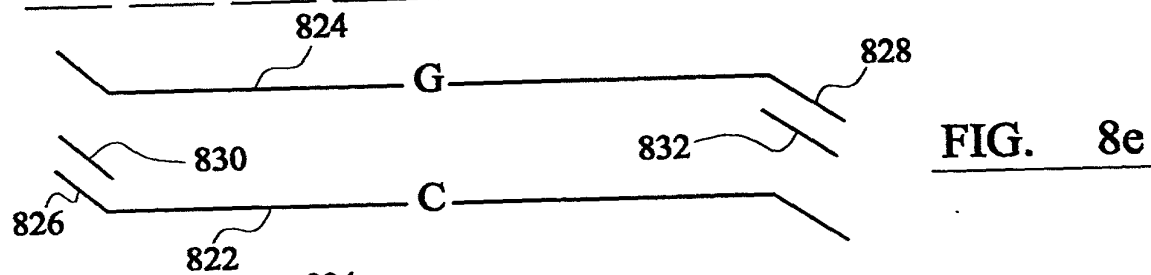
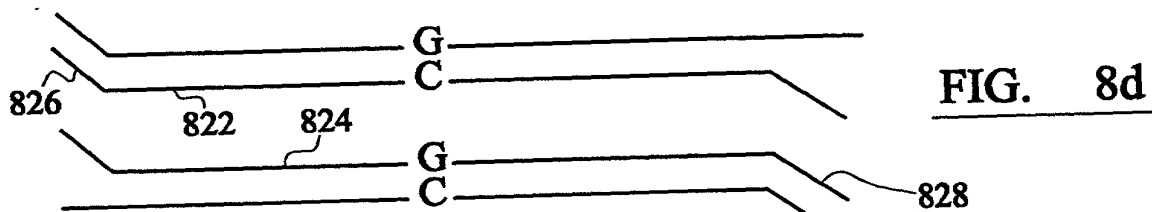
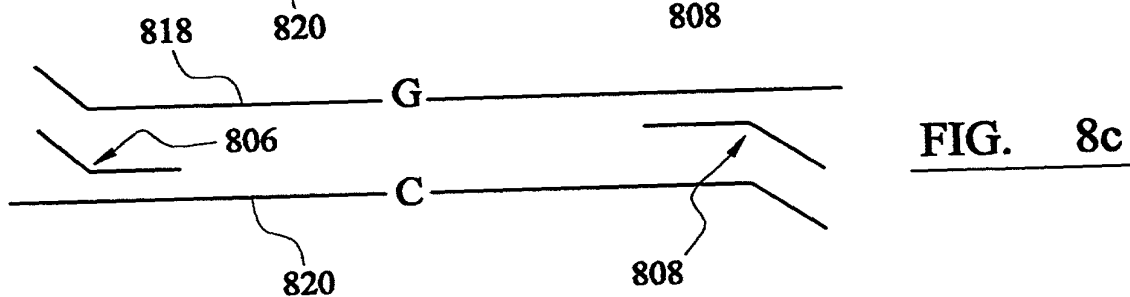
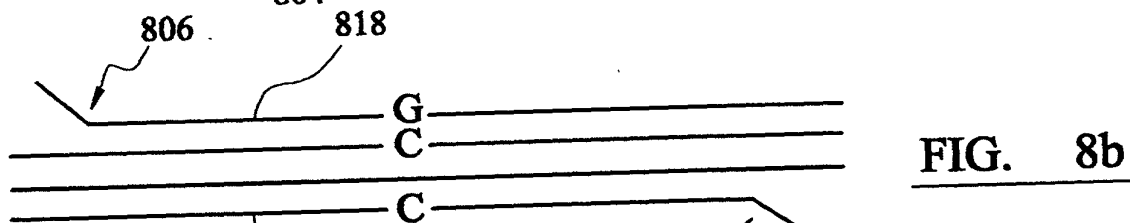
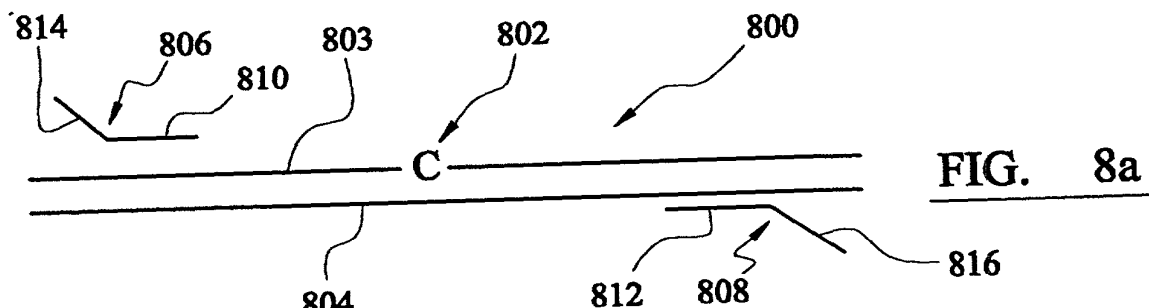


FIG. 7d

10034692-122704



100459 269400T

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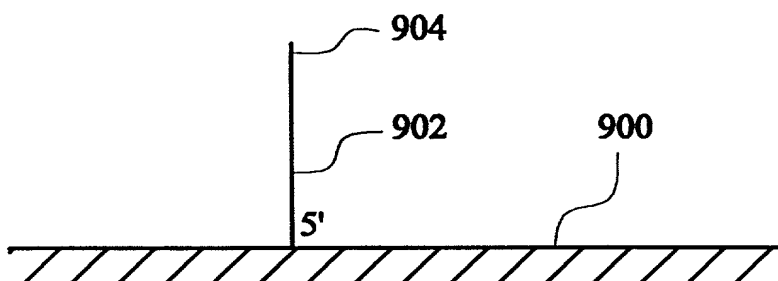


FIG. 9a

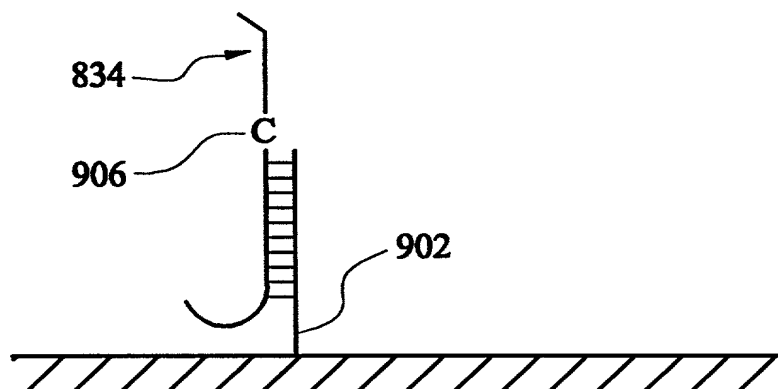


FIG. 9b

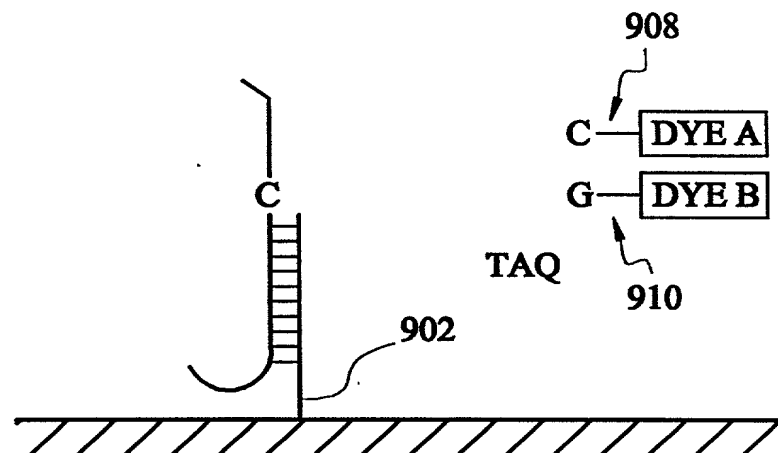


FIG. 9c

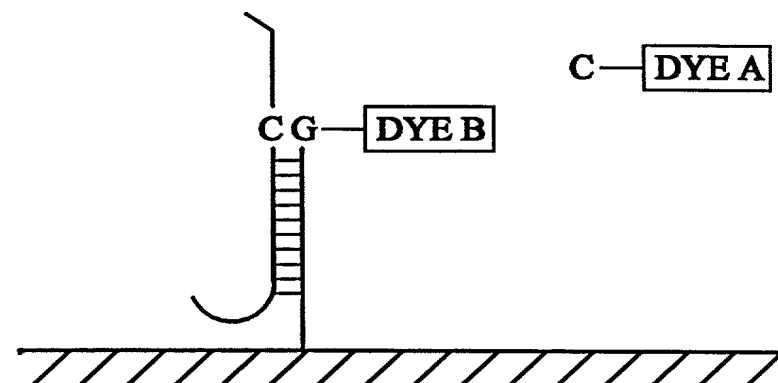


FIG. 9d

FIG. 9a

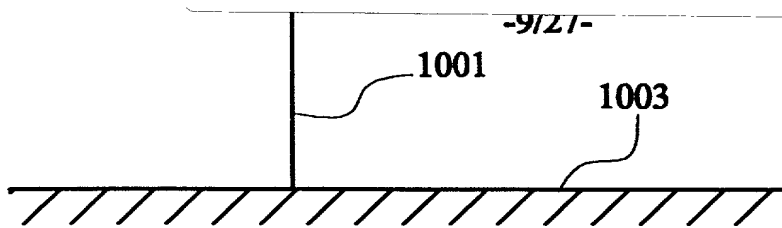


FIG. 10a

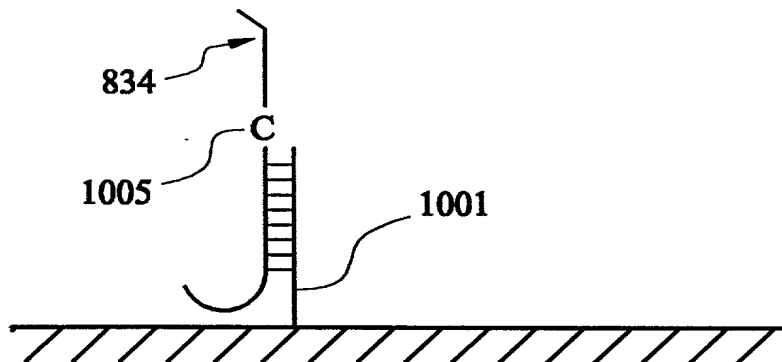


FIG. 10b

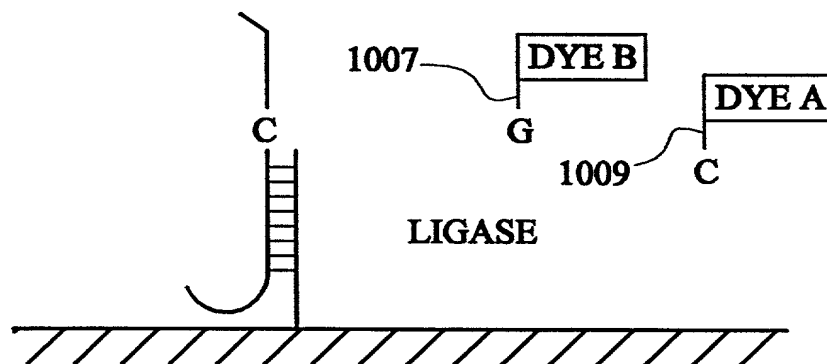


FIG. 10c

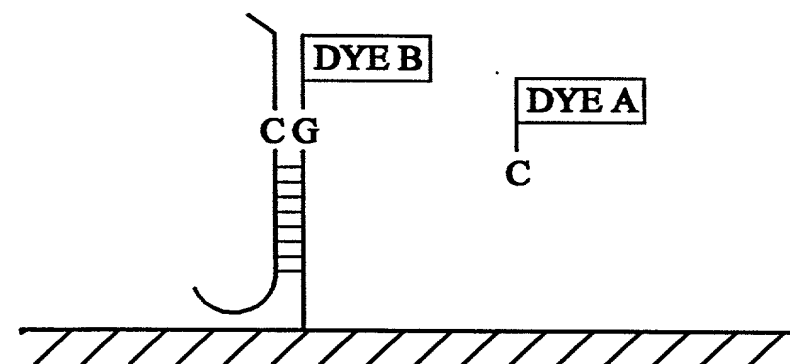


FIG. 10d

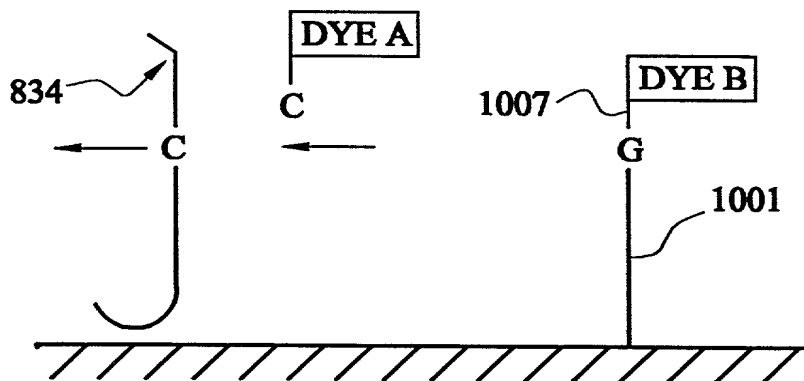


FIG. 10e

10034692.1-2694E007

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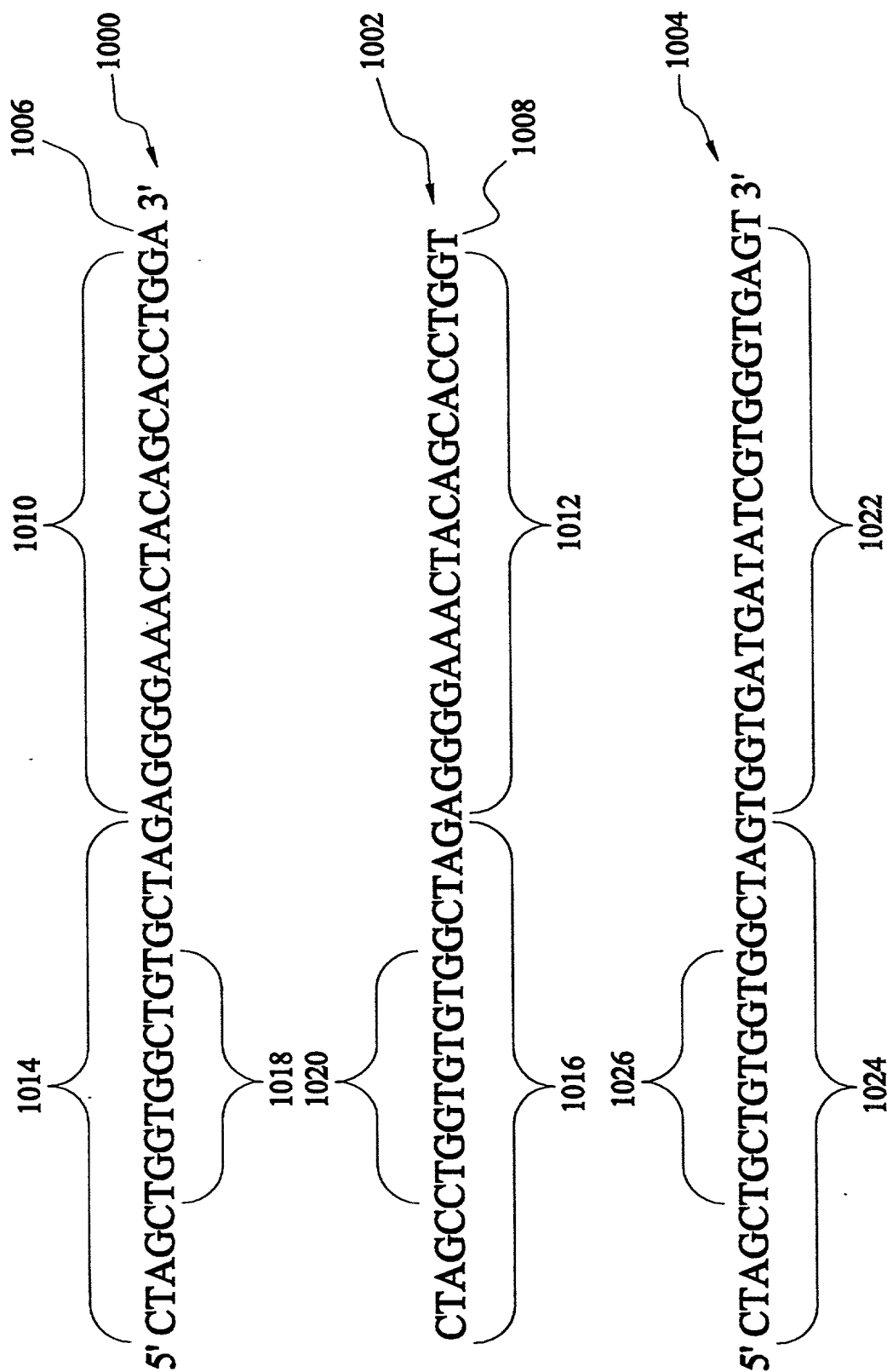


FIG. 11

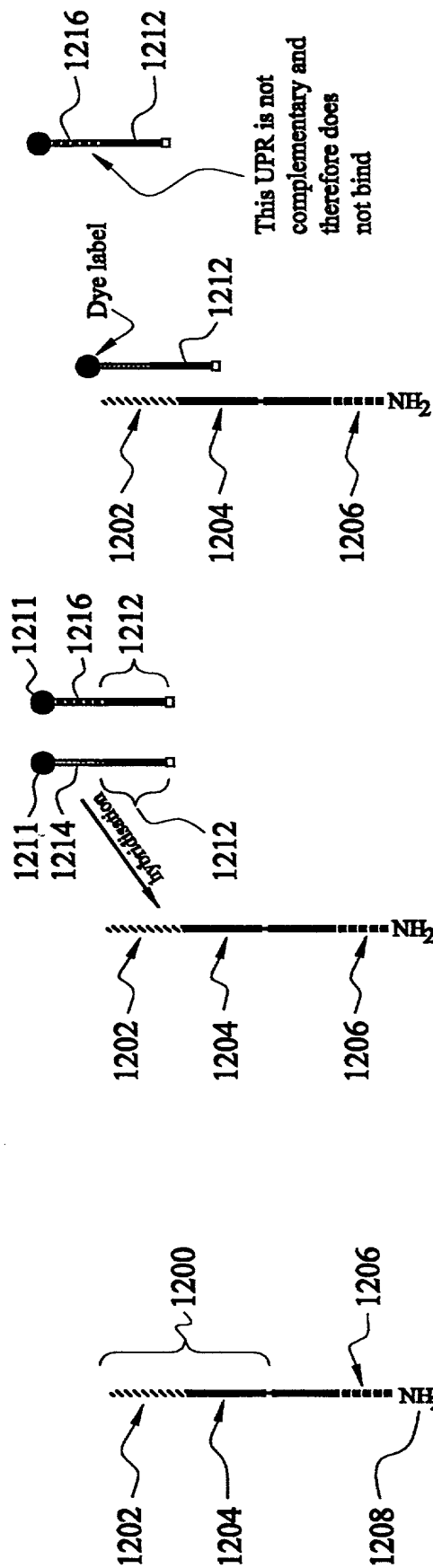


FIG. 12b



FIG. 12a



FIG. 12c



FIG. 12e



FIG. 12d



FIG. 12e



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d

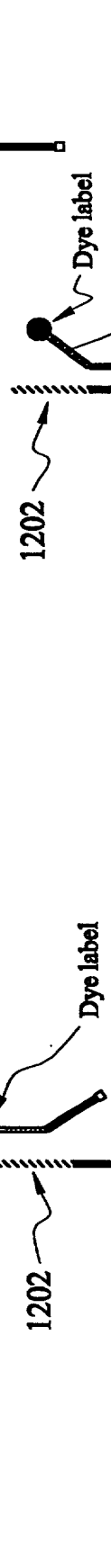


FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d

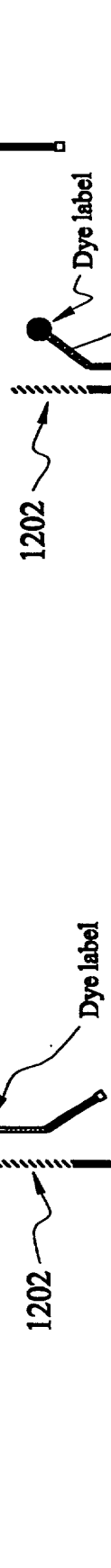


FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d

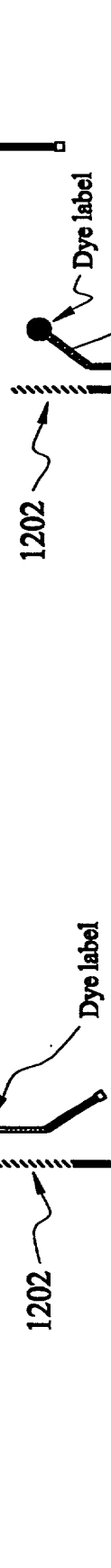


FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d



FIG. 12d

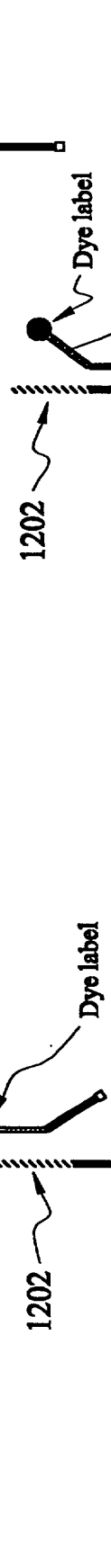


FIG. 12d



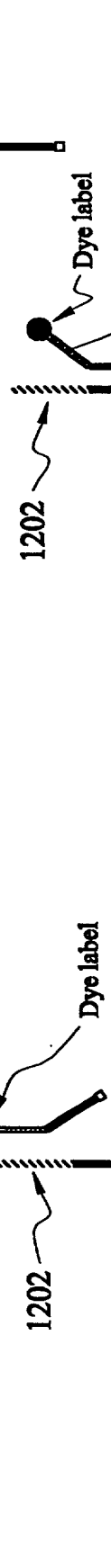
FIG. 12d



FIG. 12d



FIG. 12d



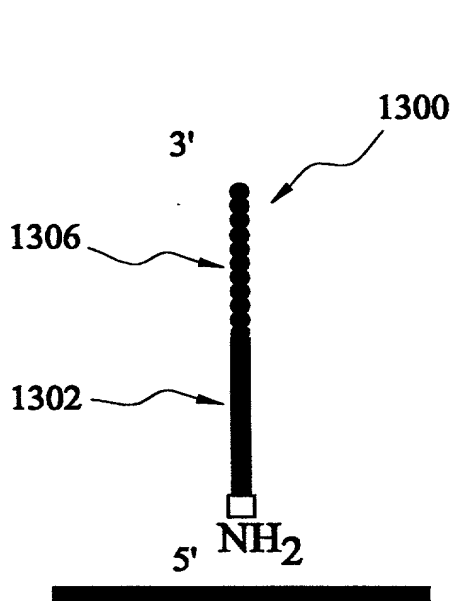


FIG. 13a

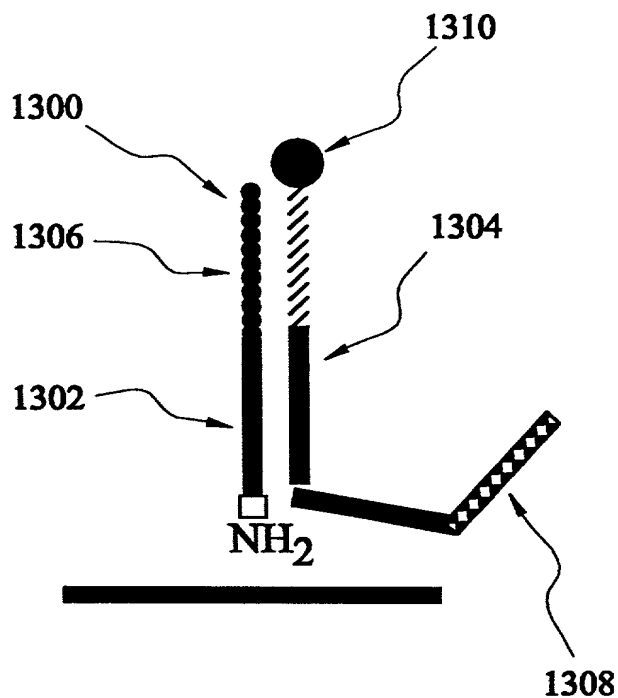


FIG. 13b

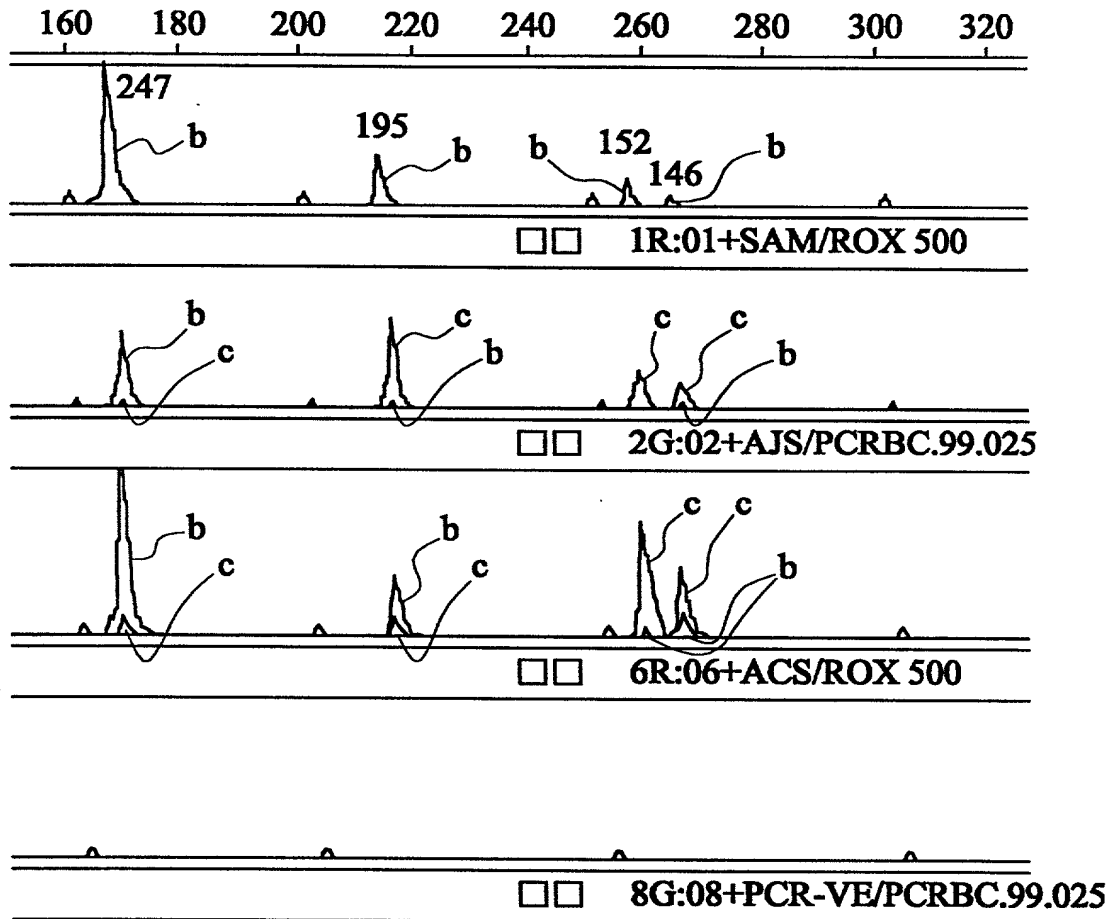
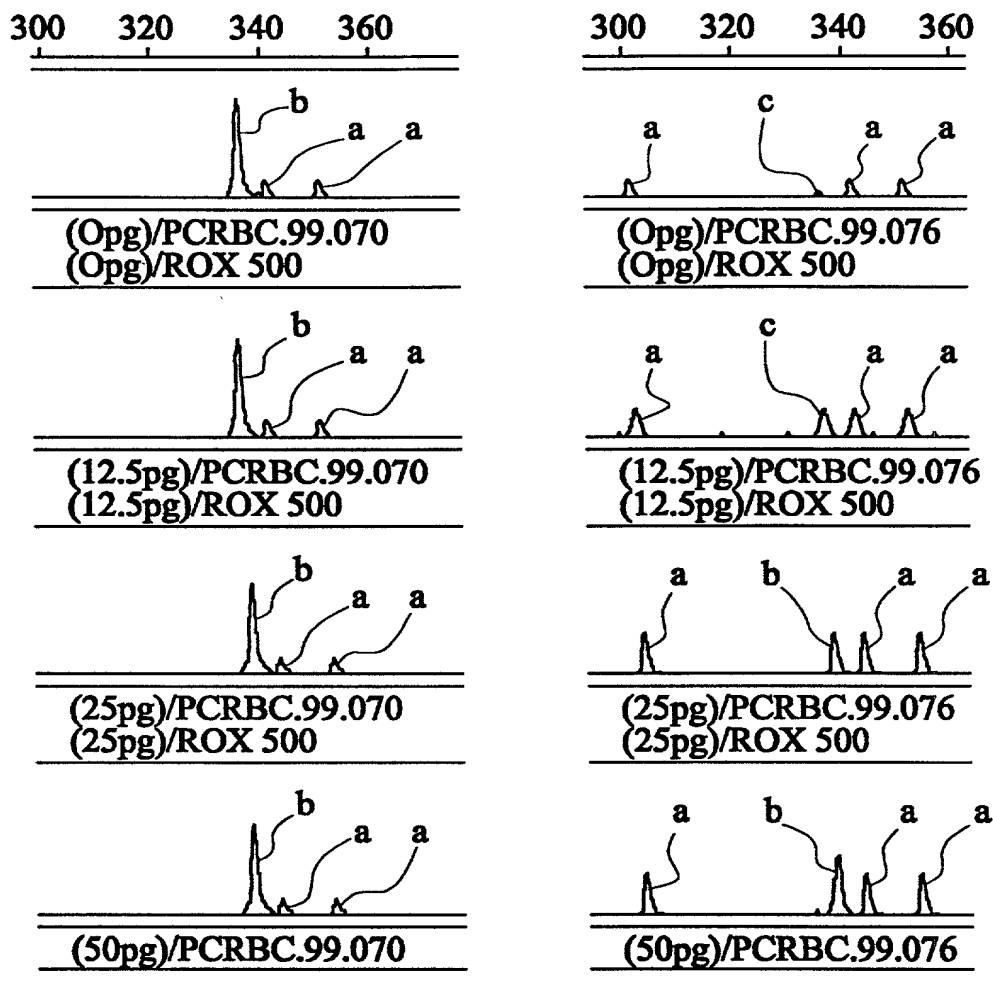


FIG. 14



Demonstration of amplification specificity and sensitivity of detection of mitochondrial DNA (a) left hand series shows detection of the major component of the mixture coding for the mt0073G polymorphism, whereas the right hand series shows detection of the minor component of the mixture, coding for the mt0073A polymorphism.

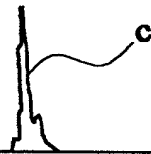
The sensitivity of the test is c. 12.5pg genomic DNA. The red peaks 'a' are standard size; the peaks 'b' are green; and the peaks 'c' are blue.

FIG. 15

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80 90 100 110 120 130 140

□□ 6G:11+1S - 1S/



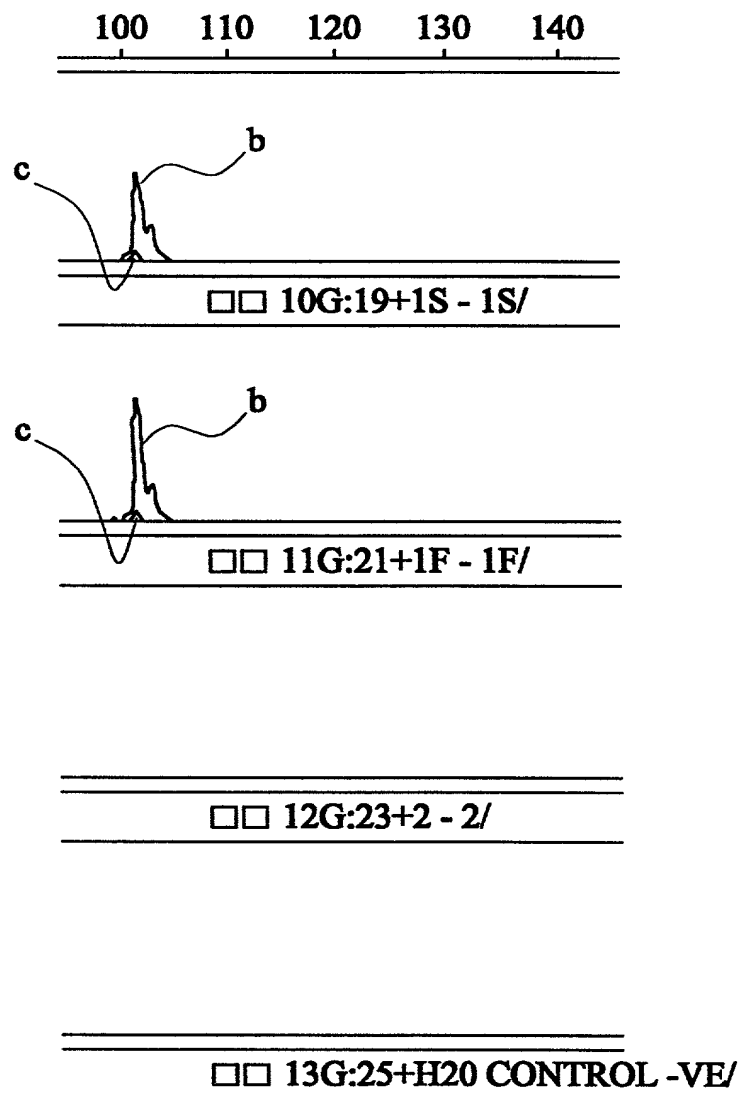
□□ 7G:13+1F - 1F/

□□ 0G:15+2 - 2/

□□ 9G:17+H2O CONTROL -VE/

Primer 416A tested against Gc 1S - 1S; 1F - 1F; 2 - 2 and negative control. Only the 1F - 1F gives a signal. No background was observed with other samples.

FIG. 16a



Singleplex reaction to test specificity of the forward primer 420G. This detects both Gc 1 polymorphisms. The primer was tested against a series of individuals - Gc 1S - 1S; 1F - 1F; 2 - 2 and a negative control. Only the first two samples gave a signal, the remainder were clear.

FIG. 16b

100 110 120 130 140

□□ 14G:27+1S - 1S/

□□ 15G:29+1F - 1F/

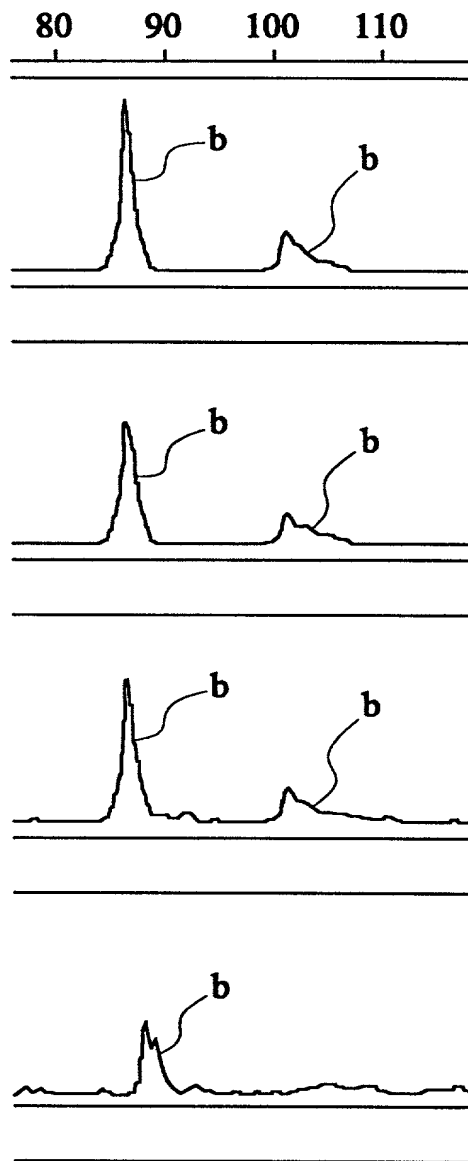
□□ 16G:31+2 - 2/

□□ 17G:83+H2O CONTROL -VE/

Singleplex reaction to test specificity of the forward primer 420T which detects Gc2. Samples are from 1S - 1S; 1F - 1F; 2 - 2 and negative control. No background was detected.

FIG. 16c

-18/21-



Demonstration of the limits of detection. A dilution series of a 1S - 1S individual was prepared (1ng, 200pg, 40pg, 8pg, respectively). A cocktail of Gc 420; Gc416 primers and the universal primers were used in the PCR reaction. Both peaks are green, indicating priming by 416C and 420G only. This is consistent with the known phenotype of the individual. No background from 416A or 420T was observed.

FIG. 16d

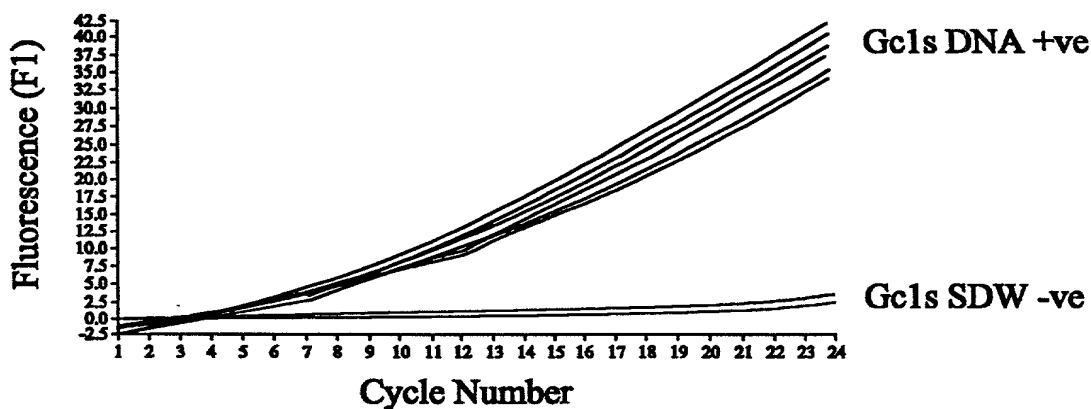


FIG. 17

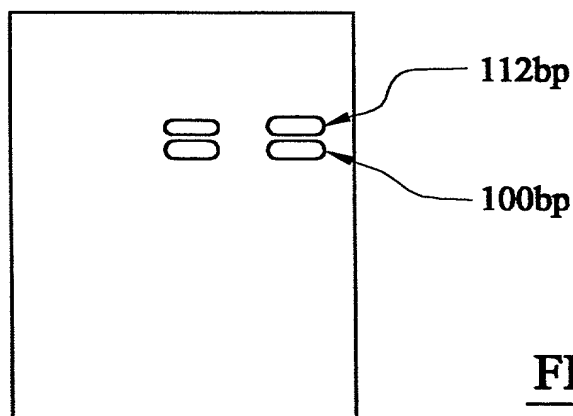


FIG. 18

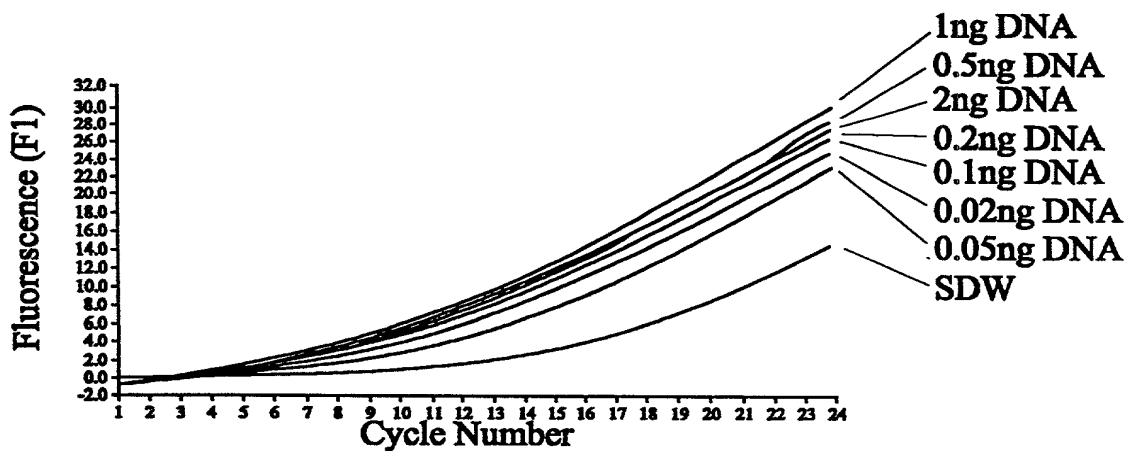
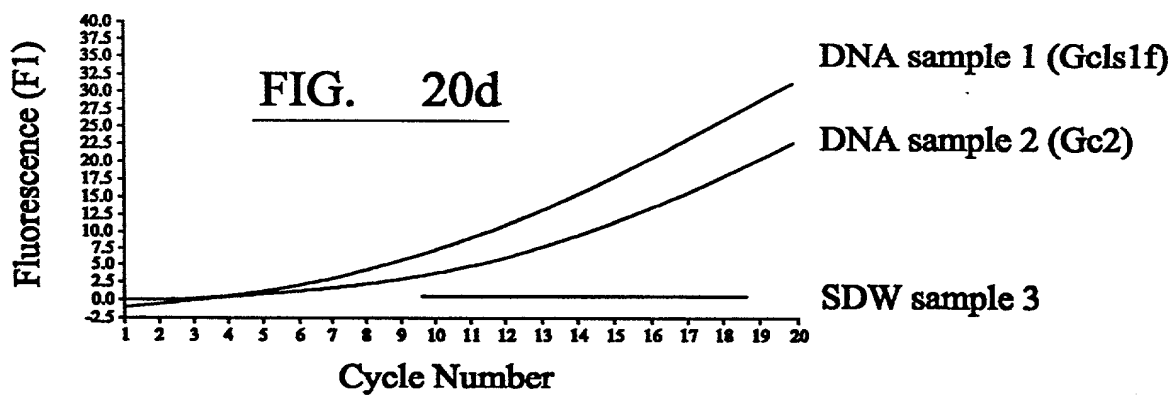
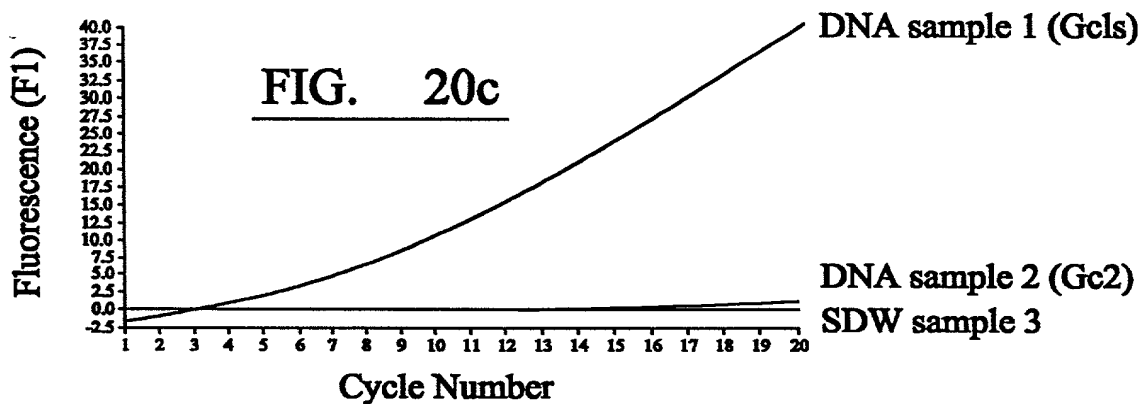
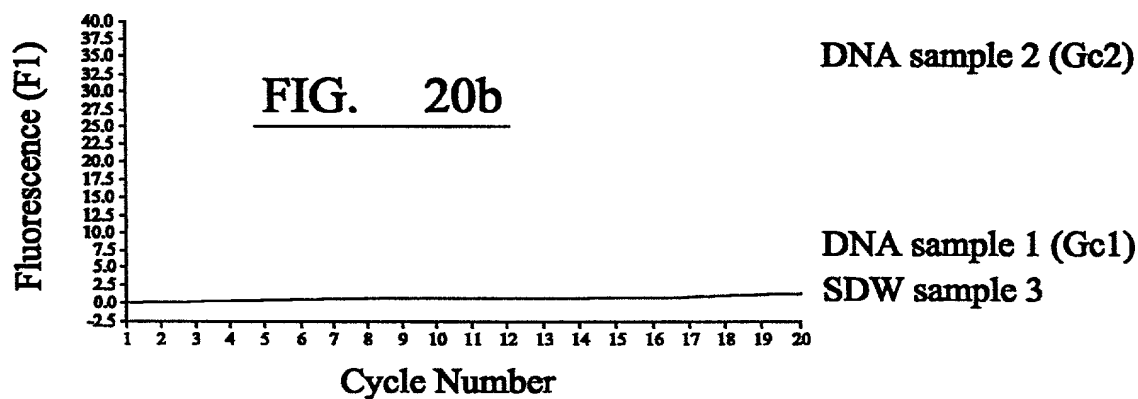
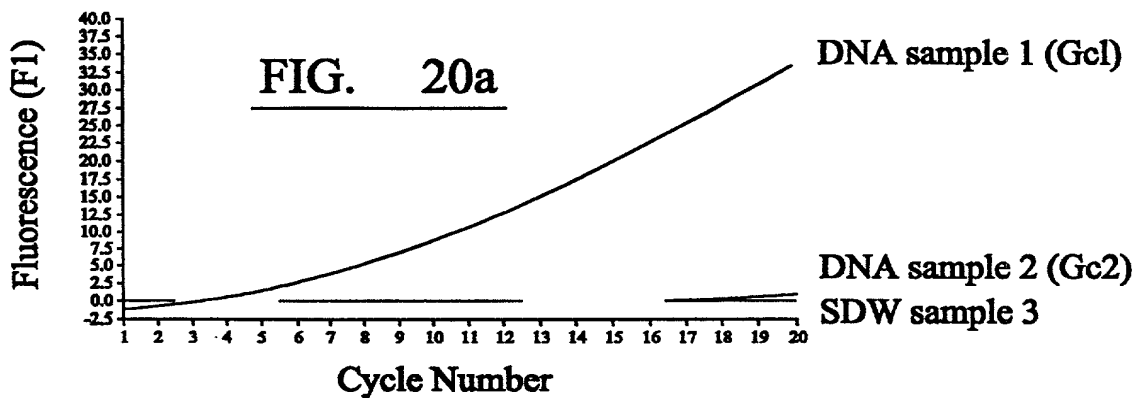


FIG. 19



416

420

	CCTGAAGCCACACCCACGGA	ACTGGCA	Gc1s DNA
NO AMPLIFICATION	CCGGTGTGGGTGCCTTGA		Gc1f primer
	CCTGAGGCCACACCCACGGA	ACTGGCA	Gc1f DNA
SPECIFIC AMP.	←CCGGTGTGGGTGCCTTGA		Gc1f primer
	CCTGAGGCCACACCCAAGGA	ACTGGCA	Gc2 DNA
NON-SPECIFIC AMP.	←CCGGTGTGGGTCCCTTGA		Gc1f primer

FIG. 21

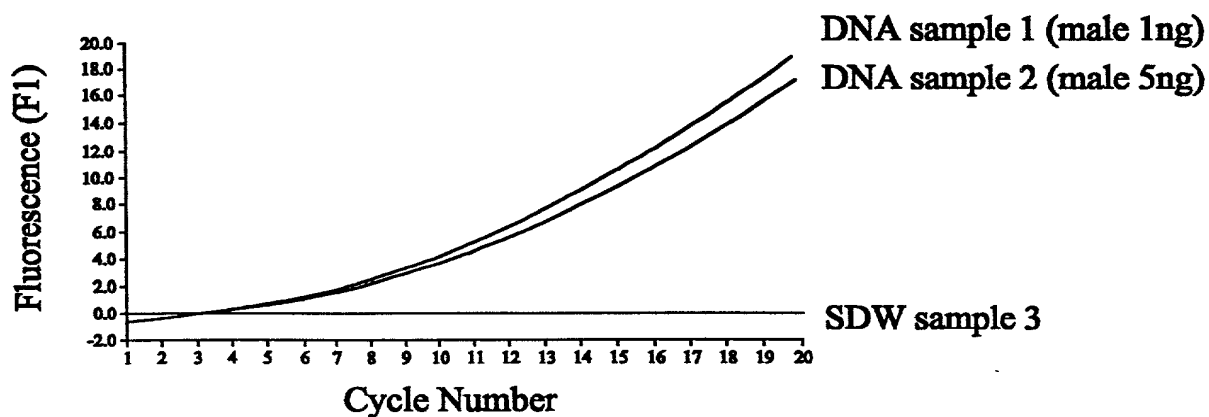


FIG. 22

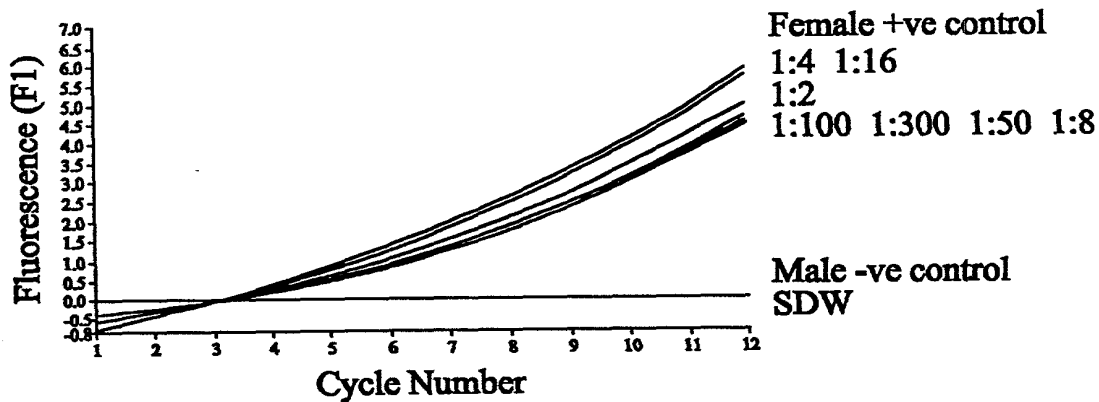


FIG. 23a

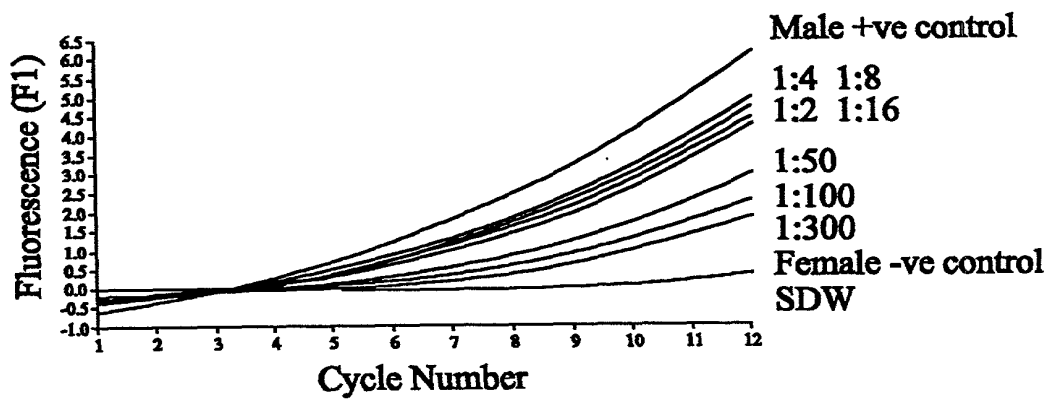


FIG. 23b

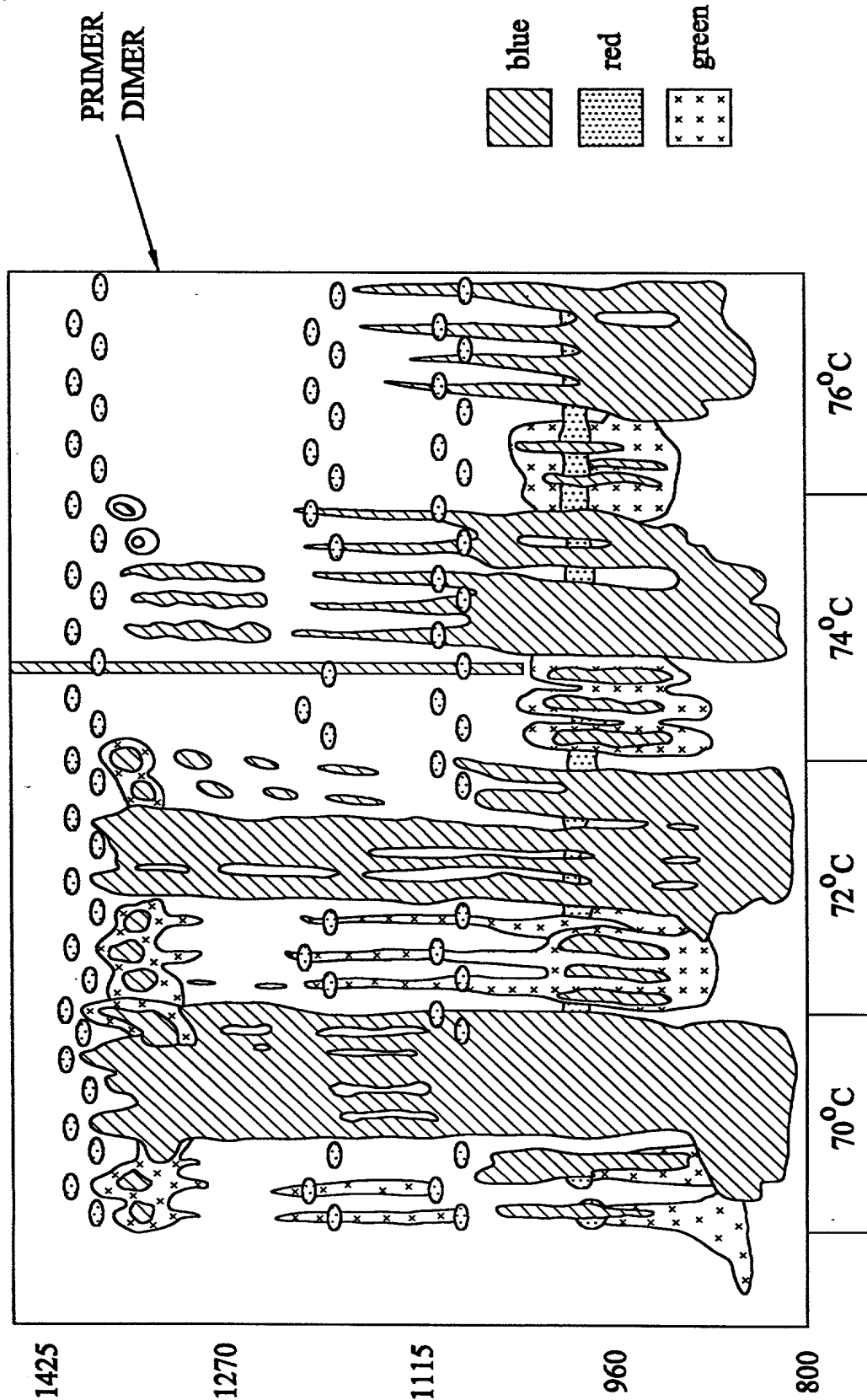


FIG. 24

A) PCR can occur with 5' overhangs

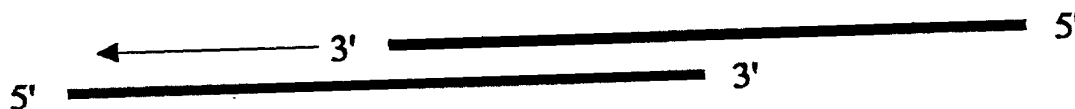


FIG. 25a

B) PCR cannot occur with 3' overhangs

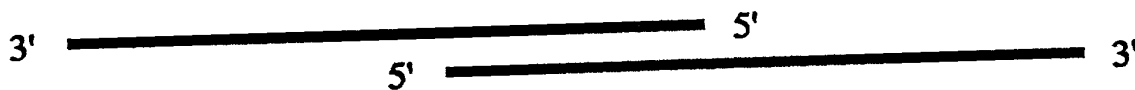
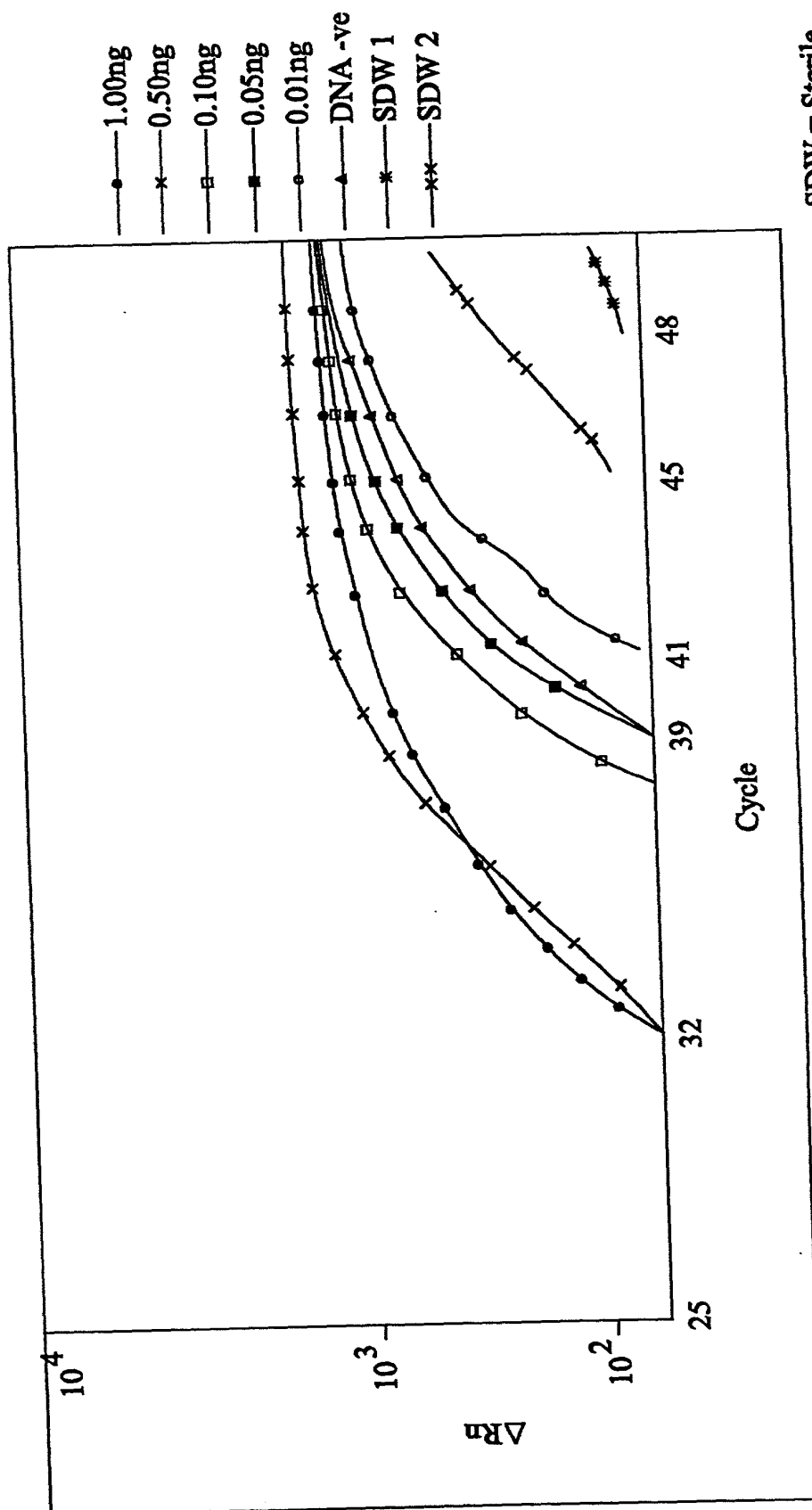


FIG. 25b

5' CTAGCTGGTGGCTGTGCTAG 3'
3' GATCGTGTCGGTGGTCGATC5'

FIG. 25c



SDW = Sterile
distilled
water

FIG. 26

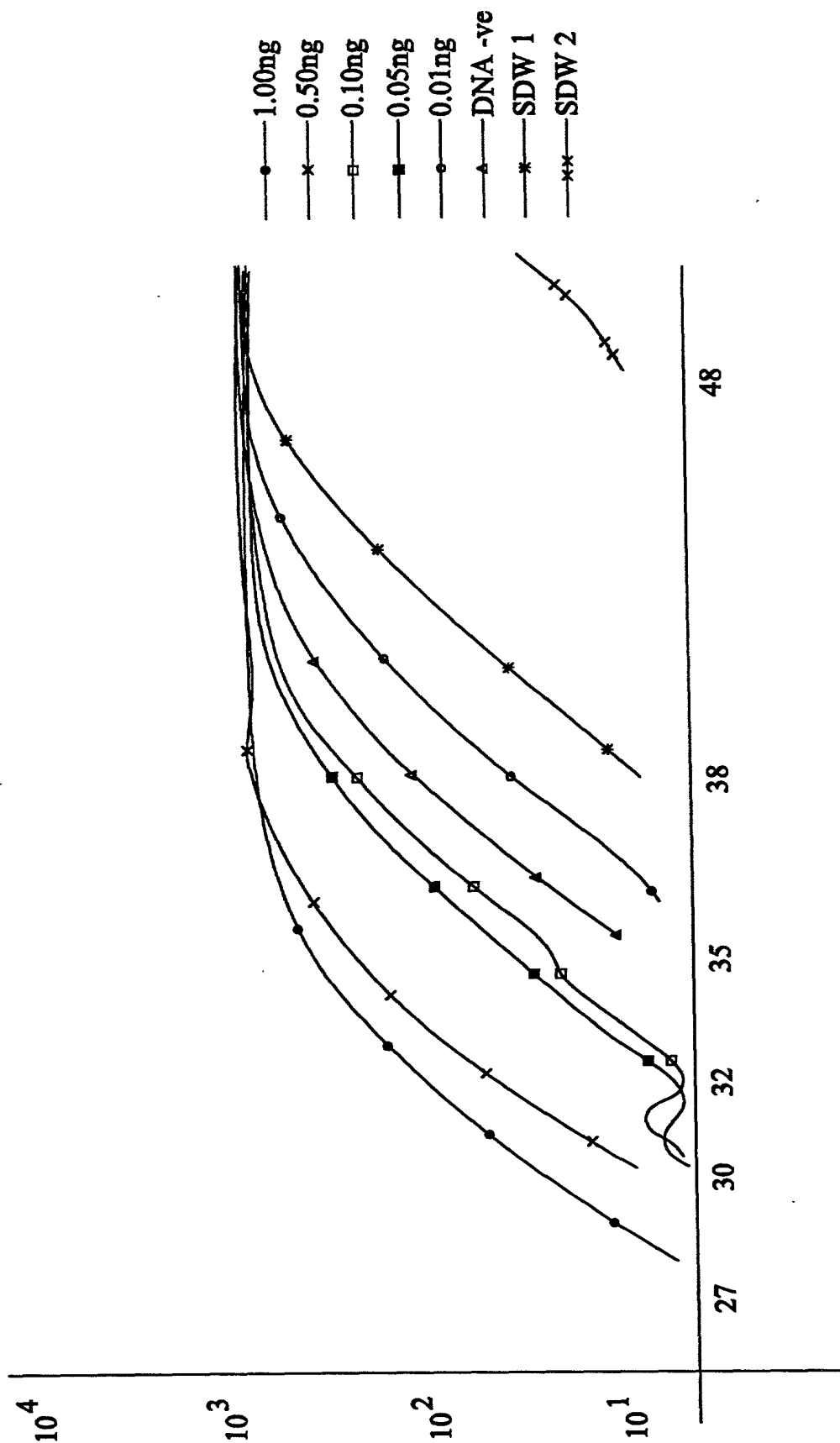


FIG. 27

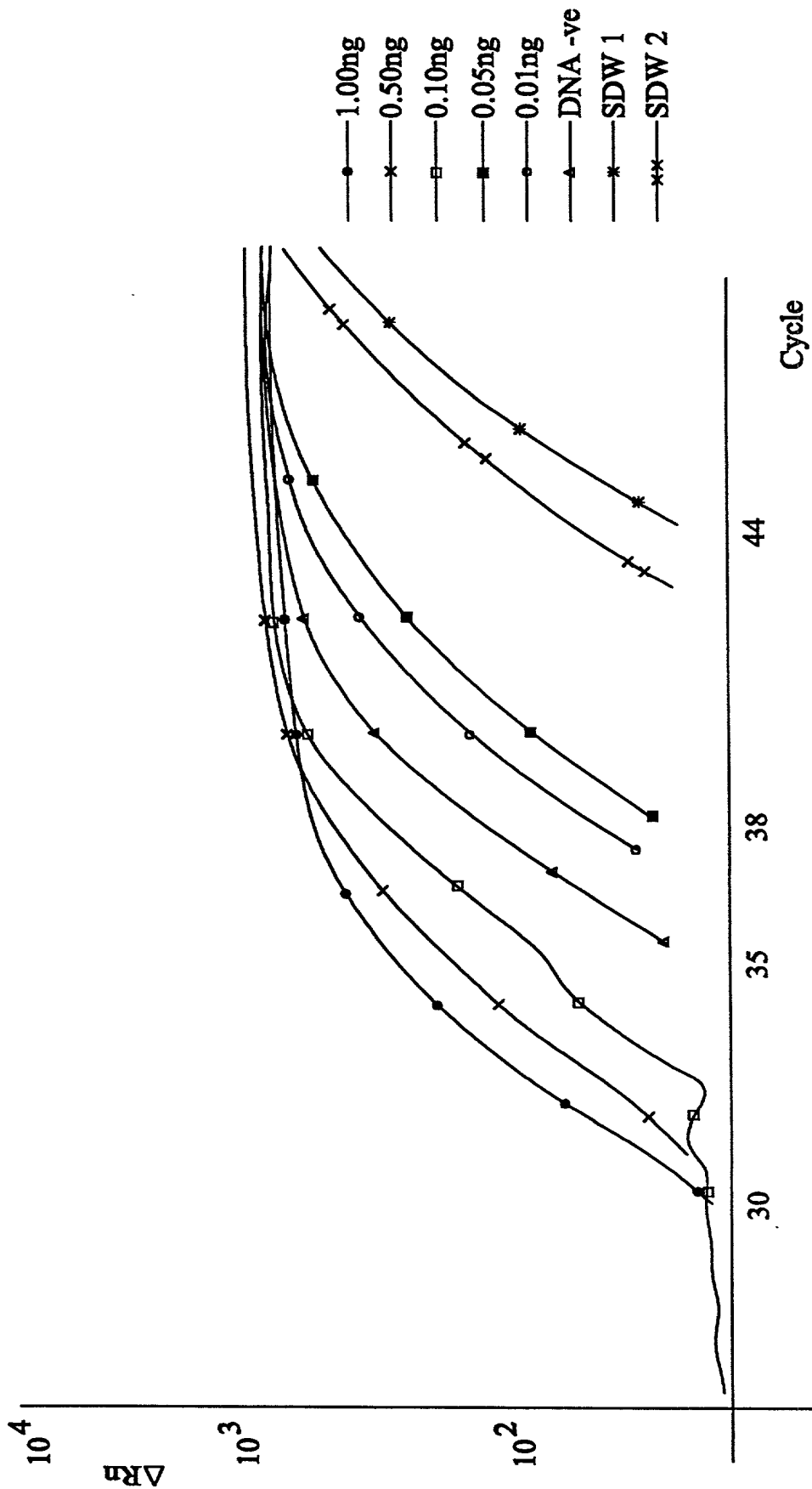


FIG. 28